Paper #63
Amdt C
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BD1 CIP FWC IV

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Sherie L. Morrison et al.
Serial No.	:	08/266,154
Filed	:	June 27, 1994
For	:	METHODS FOR PRODUCING FUNCTIONAL IMMUNOGLOBULIN, INCLUDING CHIMERIC IMMUNOGLOBULIN, IN TRANSFORMED MAMMALIAN LYMPHOCYTIC CELLS
Group Art Unit	:	1806
Examiner	:	Julie E. Reeves, Ph.D.

Official
4 May 98
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Hon. Assistant Commissioner
for Patents
Washington, D.C. 20231

May 4, 1998

do not enter
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4 May 98

AMENDMENT AFTER ALLOWANCE
PURSUANT TO 37 C.F.R. 1.312(a)

Sir:

Applicants request approval under Rule 312(a) for entry of the following amendment without withdrawing the case from issue.

IN THE CLAIMS

Please add the following claims 126-135.

126. A method for producing a functional antigen-binding protein comprised of specifically associated peptide chains, each chain derived from an immunoglobulin, which comprises the steps of:

(a) transfecting a transformed mammalian lymphocytic cell with a first DNA molecule coding for a first chain of the protein;

(b) transfecting the cell with a second DNA molecule, said second DNA molecule coding for a second chain of the protein, said second chain being a chain other than the first chain; and

(c) maintaining the cell in a nutrient medium, so that the cell expresses the first and second DNA molecules and the resultant chains are intracellularly assembled together to form the protein which is then secreted in a form capable of specifically binding to antigen

wherein prior to step (a) the cell does not express a functional antigen-binding protein.

127. A method as recited in claim 126 wherein the cell does not endogenously produce any immunoglobulin chains.

128. A method as recited in claim 126 wherein prior to step (a) the cell endogenously produces an immunoglobulin light chain or an immunoglobulin heavy chain, but not both.

129. A method as recited in claim 126 wherein each chain has a variable and a constant region.

130. A method as recited in claim 129 wherein the variable region is found in a first mammalian species and the constant region is found in a second

mammalian species, said second mammalian species being other than the first mammalian species.

131. A method for producing a functional antigen-binding protein comprised of specifically associated peptide chains, each chain derived from an immunoglobulin, which comprises the steps of:

(a) transfecting a transformed mammalian lymphocytic cell with a plasmid comprising a first DNA molecule coding for a first chain of the protein and a second DNA molecule coding for a second chain of the protein, said second chain being a chain other than the first chain; and

(b) maintaining the cell in a nutrient medium so that the cell expresses said first DNA molecule and said second DNA molecule and the resultant chains are intracellularly assembled together to form the protein which is then secreted in a form capable of specifically binding to antigen

wherein prior to step (a) the cell does not express a functional immunoglobulin capable of specifically binding antigen.

132. A method as recited in claim 131 wherein the cell does not endogenously produce any immunoglobulin chains.

133. A method as recited in claim 131 wherein prior to step (a) the cell endogenously produces an immunoglobulin light chain or an immunoglobulin heavy chain, which endogenously-produced heavy chain is not secreted in a form capable of specifically binding to antigen, but not both.

134. A method as recited in claim 131 wherein each chain has a variable and a constant region.

135. A method as recited in claim 134 wherein the immunoglobulin comprises the variable region found in a first mammalian species and comprises the constant region found in a second mammalian species, said second mammalian species being other than the first mammalian species.

REMARKS

To produce functional antigen-bonding proteins in accordance with the present invention, the specification explains that there must be a "variable region," which is the region involved with ligand or antigen binding. The antigen-binding protein must have at least one multi-subunit binding site formed from different peptide chains that are held together through specific association. The specification also explains that the "constant region" of an immunoglobin is not involved in antigen or ligand binding. The constant regions is, however, involved in functions such as complement fixation. Thus, there may be situations where it will be desirable to have a constant region, e.g., when complement fixation is desired (p. 1, lines 25-32; p.3, lines 16-23 and 29-34; and p. 4, lines 8-9.)

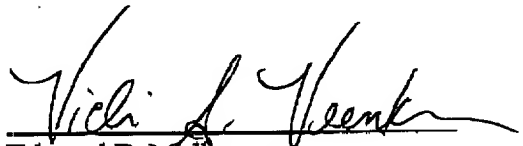
Thus, to produce a functional antigen-binding protein that achieves antigen or ligand binding in accordance with the present invention, each peptide chain must include a variable region. To also achieve functions such as complement fixation, the "heavy" chains must include constant regions.

The applicant's invention includes a method using DNA molecules that code for less than the entirety of a heavy chain. To produce a functional binding site, only the variable regions of the light and heavy chains are needed. When functions such as

complement fixation are desired, it is also necessary to include the regions that code for the constant region. Indeed, the specification teaches that even when a constant region is included, a full sequence is not required, i.e., the constant domain usually includes at least 80% of the constant region sequence (p. 10, lines 35-37).

Applicants respectfully request consideration of this Amendment prior to the May 18, 1998 deadline for payment of the issue fee and request an interview to assist the Examiners in considering the proposed claims.

Respectfully submitted,



Edward F. Mullowney
Registration No. 27,459
Vicki S. Veenker
Registration No. 34,269
Attorney for Applicants

c/o FISH & NEAVE
1251 Avenue of the Americas
New York, New York 10020-1104
Tel.: (212) 596-9000

CHIMERIC RECEPTORS BY DNA SPLICING AND EXPRESSION

This application is a continuation-in-part of U.S. Application Serial No. 644,473, filed August 27, 1984.

BACKGROUND OF THE INVENTION

Field of the Invention

Naturally occurring receptors, such as immunoglobulins, enzymes, and membrane proteins have seen an extraordinary expansion in commercial applications over the last decade. With the advent of monoclonal antibodies, the usefulness of immunoglobulins has been greatly expanded and in many situations has greatly extended prior uses employing polyclonal antibodies. However, in many applications, the use of monoclonal antibodies is severely restricted where the monoclonal antibodies are to be used in a physiological (in vivo) environment. Since, for the most part, monoclonal antibodies are produced in rodents, e.g., mice, the monoclonal antibodies are immunogenic to other species.

While the constant regions of immunoglobulins are not involved in ligand binding, the constant regions do have a number of specific functions, such as complement binding, immunogenicity, cell receptor binding, and the like. There will, therefore, be situations where it will be desirable to have constant regions which bind to cells or proteins from a particular species having binding regions for a particular ligand.

Relevant Literature

Kwan et al., J. Exp. Med. (1981) 153:1366-1370 and Clarke et al., Nucl. Acids Res. (1982) 10:7731-7749 describe V_H and V_K exons from the mouse phosphocholine-binding antibody-producing S107 myeloma cell line. Oi et al., Proc. Natl. Acad. Sci. USA (1983) 80:825-829, report that the mouse light chain gene is not expressed efficiently in a rat myeloma cell.

SUMMARY OF THE INVENTION

Chimeric multi-subunit receptors are provided, where each of the subunits is an expression product of a fused gene. Each fused gene comprises a DNA sequence from one host species encoding the region involved with ligand binding joined to a DNA sequence from a different source, either the same or a different host species, encoding a "constant" region providing a structural framework and biological properties. Introduction of the fused genes into an appropriate eukaryotic host cell under conditions for expression and processing provides for a functional assembled multi-subunit receptor product.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic diagram of (a) the chimeric mouse:human heavy chain gene vector; and (b) the chimeric light chain vector.

Figure 2 is a schematic diagram of chimeric human IgG anti-DNS expression vectors.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Novel methods and compositions are provided, for production of polypeptide products having specific binding affinities for a predetermined ligand and predetermined biological, particularly physiological, properties, each of which are not normally associated with the binding region peptide sequences. Particularly,

multi-subunit chimeric receptors are provided which result from fused genes having the portion of the polypeptide involved with binding of a predetermined ligand having an amino acid sequence substantially the same (>90% conserved) as an amino acid sequence having the same function from one host, while the portion involved with providing structural stability, as well as other biological functions, being analogously derived from a different host. The resulting composition can be either an inter- or intraspecies chimera. At least two fused genes are involved, which genes are introduced into an appropriate eukaryotic host under conditions for expression and processing, whereby the fused genes are expressed and the resulting subunits bound together, resulting in an assembled chimeric receptor.

The receptors prepared in accordance with the subject invention will be multi-subunit, where the units are held together either by non-covalent binding or a combination of non-covalent and covalent binding, particularly disulfide linkages through cysteine, and having at least one binding site, usually at least two binding sites, and not more than about ten binding sites. Receptors of interest include both B-cell and T-cell receptors, more particularly, immunoglobulins, such as IgM, IgG, IgA, IgD and IgE, as well as the various subtypes of the individual groups. The light chain may be κ or λ. The heavy chains are referred to as μ, γ, α, δ, and ε.

In discussing the two regions of each subunit, the two regions will be referred to as "variable" and "constant" by analogy to immunoglobulins. The variable region is the region involved with ligand binding and, therefore, will vary in conformation and amino acid sequence depending upon the ligand. The region will usually be composed of a plurality of smaller regions (hypervariable or complementary determining regions), involving a region having as its primary function bind-

ing to the ligand (V) and a region associated with joining the V region to the constant region, the joining region (J). There may also be a hypervariable region joining the V and J regions, the diversity region (D). These regions are related to gene segments observed in the genes encoding immunoglobulin variable regions.

The constant region will not be associated with ligand binding and will be relatively limited in the variations in its conformation and amino acid sequence within any one species and within any one class, each class generally having from 1 to 4 subclasses. Each constant region is specific for a species. Within the classes there will be allotypes, individual polymorphisms within a class within a species.

The varible region of the immunoglobulins will be derived from a convenient mammalian source, which may be a rodent, e.g., mouse or rat, rabbit, or other vertebrate, mammalian or otherwise, capable of producing immunoglobulins. The constant region of the immunoglobulin, as well as the J chain for IgM and IgA (not the same as the J region of the heavy or light immunoglobulin chain), will be derived from a vertebrate source different from the source of the variable region, particularly a mammalian source, more particularly primate or domestic animal, e.g., bovine, porcine, equine, canine, feline, or the like, and particularly, humans. The different source of the constant region can be either from a different species or from the same species as the mammalian source utilized to provide the variable region. Thus, the constant region of the receptor will normally be chosen in accordance with the purpose of the receptor. For example, where the receptor is to be introduced into the host, the constant portion will be selected so as to minimize the immune response of the host to the receptor and to optimize biological efficiency, such as complement fixation or

physiologic[redacted] half-life (catabolism). [redacted]ere the receptor is to bind to particular cell membrane surface receptors, the constant region will be chosen in accordance with the host of the receptor recognition site.

The fused gene derived from the two host sources will be prepared by joining the 5'-end of a sequence encoding the constant region in reading frame to the 3'-end of a sequence encoding the variable region. (In referring to 5' or 3' for a double strand, the direction of transcription is with 5' being upstream from 3'.) With immunoglobulins, two fused genes will be prepared, one for the light chain and one for the heavy chain. With T-cell receptors, the two fused genes will be for each of the two chains involved in the formation of the T-cell receptor. The DNA sequences employed for prepartion of the fused gene may be derived from a variety of sources. These sources include genomic DNA, cDNA, synthetic DNA, and combinations thereof. The genomic DNA may or may not include naturally occurring introns.

The DNA obtained from natural sources, namely the genomic DNA or cDNA, may be obtained in a variety of ways. Host cells coding for the desired sequence may be isolated, the genomic DNA may be fragmented, conveniently by one or more restriction endonucleases, and the resulting fragments may be cloned and screened with a probe for the presence of the DNA sequence coding for the polypeptide sequence of interest. For the variable region, the rearranged germline heavy chain DNA will include V, D, and J regions, including the leader sequence, which may be subsequently removed as well as any introns. The rearranged germline light chain coding DNA will include the V and J regions including the leader sequence, as well as any introns which may be subsequently removed. The particular source of the exons defining the domains and the manner of splicing, where introns are present, is not germane

to this invention. Once the cloned fragment has been identified which contains the desired DNA sequence, this fragment may be further manipulated to remove superfluous DNA, modify one or both termini, remove all or a portion of intervening sequences (introns), or the like.

In providing a fragment encoding the variable region, it will usually be desirable to include all or a portion of the intron downstream from the J region. Where the intron is retained, it will be necessary that there be functional splice acceptor and donor sequences at the intron termini. The gene sequence between the J (joining region) and the constant region of the fused gene may be primarily the intron sequence associated with (1) the constant region, (2) the J region, or (3) portions of each. The last may be a matter of convenience where there is a convenient restriction site in the introns from the two sources. In some instances, all or a portion of the intron may be modified by deletion, nucleotide substitution(s) or insertion, to enhance ease of manipulation, expression, or the like. When the variable region is chosen to be syngeneic with the host cells employed for expression, all or at least about 80% of the intron sequence can be selected from the naturally occurring intron sequence associated with the J region. In some instances it will be necessary to provide adapters to join the intron or truncated intron to the constant region. By cleaving within the intron, the variable region will be separated from its natural constant region.

Alternatively, it may be desirable to have the fused gene free of the intron between the variable and constant regions. Thus, the 3' terminus will be at or in the joining region. Normally all or a portion of the J region will be associated with the host providing the variable region. By restriction enzyme analysis or sequencing of the J region, one can select for a particular site for the 3' terminus of the variable region.

Alternatively, one can use an exonuclease and by employing varying periods of digestion, one can provide for varying 3'-termini, which can then be used for linking to the constant region and selection made for a functional product in a variety of ways. For example, where joining of the variable region to the constant region results in a unique restriction site, the fused DNA fragments may be screened for the presence of the restriction site.

Alternatively, it may be found desirable to include an adapter or linker to join the variable region to the constant region, where the adapter or linker may have the same or substantially the same sequence, usually at least substantially the same sequence, of the DNA sequence of the two fragments adjacent the juncture. The adapter or linker will be selected so as to provide for the two sequences to be in common reading frame. Furthermore, by employing adapters, one could add an additional degree of variability in the binding affinity of the chimeric receptor, by providing for the expression of different amino acids in the J region.

The joining of the various fragments is performed in accordance with conventional techniques, employing blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases.

For cDNA, the cDNA may be cloned and the resulting clone screened with an appropriate probe for cDNA coding for the desired variable or constant region. Once the desired clone has been isolated, the cDNA may be manipulated in substantially the same manner as the genomic DNA. However, with cDNA there will be no introns or intervening sequences. The cDNA is

cleaved at [redacted] near the juncture of the [redacted]riable region with the constant region so that the variable region is separated from the constant region and the desired region retained. Where a convenient restriction site exists, the cDNA may be digested to provide for a fragment having the appropriate terminus. The restriction site may provide a satisfactory site or be extended with an adapter. Alternatively, primer repair may be employed, where for the variable region a complementary sequence to the site of cleavage and successive nucleotides in the 3' direction of the complementary sequence is hybridized to the sense strand of the cDNA and the nonsense strand replicated beginning with the primer and removal of the single-stranded DNA of the sense strand 3' from the primer. The reverse is true for the constant region. Other techniques may also suggest themselves. Once the fragment has been obtained having the predetermined 3' or 5' terminus, as appropriate, it may then be employed for joining to the other region.

Finally, one or both of the regions may be synthesized and cloned for use in preparing the fused gene. For the most part, the same or substantially the same constant region can be repetitively used, so that a library of constant regions may be established which can be selected for joining to variable regions. Thus, the constant regions would have an appropriate 5' terminus for joining directly or through an adapter to a variable region.

In order for expression of the fused gene, it will be necessary to have transcriptional and translational signals recognized by an appropriate eukaryotic host. For the most part, desirable eukaryotic hosts will be mammalian cells capable of culture in vitro, particularly leukocytes, more particularly myeloma cells, or other transformed or oncogenic lymphocyte, e.g., EBV transformed cells. Alternatively, non-mammalian cells may be employed, such as fungi, e.g., yeast, filamentous fungi, or the like.

The DNA sequence coding for the variable region may be obtained in association with the promoter region from genomic DNA. To the extent that the host cells recognize the transcriptional regulatory and translational initiation signals associated with the variable region, then the region 5' of the variable region coding sequence may be retained with the variable region coding sequence and employed for transcriptional and translational initiation regulation.

The contiguous non-coding region 5' to the variable region will normally include those sequences involved with initiation of transcription and translation, such as the TATA box, capping sequence, CAAT sequence, and the like. Usually the 5'-non-coding sequence will be at least 150bp, more usually at least 200bp, usually not exceeding about 2kbp, more usually not exceeding about 1kbp.

The non-coding region 3' to the constant region may be retained for its transcriptional termination regulatory sequences, such as termination and polyadenylation. Thus, by retaining the 3'-region naturally contiguous to the DNA sequence coding for the constant region, the transcriptional termination signals may be provided for the fused gene. Where the transcriptional termination signals are not satisfactorily functional in the expression host cell, then a 3' region functional in the host cell may be substituted. Conveniently, the non-coding 3' region may be obtained from a non-coding contiguous 3' region of a constant region from the expression host. The 3'-non-coding region may be joined to the constant region by any of the means described previously for manipulation and ligation of DNA fragments. This region could then be used as a building block in preparing the fused gene.

The fused gene for the most part may be depicted by the following formula:

$TIR-(\bullet)_e-V_f-(Vi)_a-(D)_b-(Di)_c-J-(\bullet)_d-C-TTR$

wherein:

TIR intends the transcriptional regulatory and translational initiation region and is generally of at least about 150bp and not more than about 2kbp, which may be in whole or in part the sequence naturally joined to the V coding region;

LS refers to a DNA sequence encoding a leader sequence and processing signal functional in the expression host for secretion and processing for removal of the sequence; this leader sequence can contain an intron, as is known in the art to occur;

e is 0 or 1;

V is a segment coding for the variable domain in reading frame with LS, when LS is present;

f is 0 or 1;

D is a segment coding for the diversity domain and is present for the heavy chain (b=1) and is absent for the light chain (b=0);

J is a segment coding for the joining region;

Vi and Di are introns associated with the letter-indicated coding segments having functional donor and acceptor splicing sites;

a, b and c are the same or different and are 0 or 1, wherein when b is 0, c is 0; a, b, and c are all preferably 0;

I is an intron which may be naturally contiguous to the J segment or naturally contiguous to the C domain or a combination of fragments from both, or a fragment thereof, desirably including an enhancer sequence functional in said expression host, or I may be foreign in whole or in part to the J and C segments;

d is 0 or 1 (preferably 0);

C is the constant domain and may code for a μ, γ, δ, α or ε chain, preferably μ, γ, or α, usually including at least 80% of the constant region sequence,

and may be the same as or a modified naturally occurring allotype or an altered constant region encoding an improved protein sequence; and

TTR is the transcriptional termination region providing for transcriptional termination and polyadenylation which may be naturally associated with C or may be joined to C, being functional in the expression host; usually being at least about 100bp and may be 1kbp or more.

Fused genes lacking, or containing modifications in, the hinge region or other immunoglobulin constant region domains can also be prepared, in like manner to the modifications described above, in which case the formula will be as shown above but with the hinge region of the constant chain being absent or modified.

The constructs for each of the different subunits may be joined together to form a single DNA segment or may be maintained as separate segments, by themselves or in conjunction with vectors.

The subunit constructs may be introduced into a cell by transformation in conjunction with a gene allowing for selection where the construct will become integrated into the host genome.

A large number of vectors are available or can be readily prepared which provide for expression in a host, either by maintenance as an extrachromosomal element or by integration into the host genome. For a mammalian host, a wide variety of vectors are known based on viral replication systems, such as Simian virus, bovine papilloma virus, adenovirus and the like. These vectors can be used as expression vectors where transcriptional and translational initiation and termination signals are present and one or more restriction sites are available for insertion of a structural gene. In addition, the vectors normally have one or more markers which allow for selection of host cells which contain the expression vector. The marker may provide for prototrophy to an auxotrophic host; biocide

Paper #63

IN RE U.S. PATENT APPLICATION
SERIAL NUMBER: 08/266,154

EXAMINER: Julie E. Reeves, Ph.D.
GROUP: 1806

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THE SENDER IS:

Vicki S. Veenker
Registration No. 34,269
c/o FISH & NEAVE
1251 Avenue of the Americas
New York, New York 10020
Tel. (650) 617-4000
Fax. (650) 617-4090

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REV. 10/97
For Other Than A Small Entity

Docket No. BD1 CIP FWC IV

Applicant(s) : Sherie L. Morrison et al.

Serial No. : 08/266,154

Filed : June 27, 1994

For : METHODS FOR PRODUCING FUNCTIONAL IMMUNOGLOBULIN, INCLUDING CHIMERIC IMMUNOGLOBULIN, IN TRANSFORMED MAMMALIAN LYMPHOCYTIC CELLS

Group Art Unit : 1806

Examiner : Julie E. Reeves, Ph.D.

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Hon. Assistant Commissioner
for Patents
Washington, D.C. 20231

May 4, 1998

TRANSMITTAL LETTER

Sir:

Transmitted herewith: [ ] a Preliminary Amendment; [X] an Amendment under 37 C.F.R. § 1.312(a); [ ] A Request for Refund; [ ] a substitute Specification; [ ] a Declaration; [ ] a Supplemental Declaration; [ ] a Power of Attorney; [ ] an Associate Power of Attorney; [ ] formal drawings; to be filed in the above-identified patent application.

FEE FOR ADDITIONAL CLAIMS

[X] A fee for additional claims is not required.

[ ] A fee for additional claims is required.

The additional fee has been calculated as shown below:

	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR		PRESENT EXTRA		RATE	ADDITIONAL FEES
TOTAL CLAIMS	40	-	48	*	= 0	X	$22 =	$ 0
INDEPENDENT CLAIMS	5	-	6	**	= 0	X	$82 =	$ 0
FIRST PRESENTATION OF A MULTIPLE DEPENDENT CLAIM						+	$270 =	$
							TOTAL	$_____

* If less than 20, insert 20.

** If less than 3, insert 3.

[ ] A check in the amount of $_____ in payment of the filing fee is transmitted herewith.

[X] The Commissioner is hereby authorized to charge payment of any additional filing fees required under 37 C.F.R. § 1.16, in connection with the paper(s) transmitted herewith, or credit any overpayment of same, to deposit Account No. 06-1075. A duplicate copy of this transmittal letter is transmitted herewith.

[ ] Please charge $_____ to Deposit Account No. 06-1075 in payment of the filing fee. A duplicate copy of this transmittal letter is transmitted herewith.

EXTENSION FEE

[ ] The following extension is applicable to the Response filed herewith; [ ] $110.00 extension fee for response within first month pursuant to 37 C.F.R. § 1.17(a);

[] $400.00 extension fee for response within second month pursuant to 37 C.F.R. § 1.17(b); [ ] $950.00 extension fee for response within third month pursuant to 37 C.F.R. § 1.17(c); [ ] $1,510.00 extension fee for response within fourth month pursuant to 37 C.F.R. § 1.17(d).

[ ] A check in the amount of [ ] $110.00; [ ] $400.00; [ ] $950.00; [ ] $1,510.00; in payment of the extension fee is transmitted herewith.

[X] The Commissioner is hereby authorized to charge payment of any additional fees required under 37 C.F.R. § 1.17 in connection with the paper(s) transmitted herewith, or to credit any overpayment of same, to Deposit Account No. 06-1075. A duplicate copy of this transmittal letter is transmitted herewith.

[ ] Please charge the [ ] $110.00; [ ] $400.00; [ ] $950.00; [ ] $1,510.00; extension fee to Deposit Account No. 06-1075. A duplicate copy of this transmittal letter is transmitted herewith.

Vicki S. Veenker

Edward F. Mullowney (Reg. No. 27,459)
Vicki S. Veenker (Reg. No. 34,269)
Attorneys for Applicants

c/o FISH & NEAVE
1251 Avenue of the Americas
New York, New York 10020-1104
Tel.: (212) 596-9000